SUPPORTING INFORMATION

Anticonvulsants Based on the α-Substituted Amide Group Pharmacophore Bind to and Inhibit Function of Neuronal Nicotinic Acetylcholine Receptors

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Table S1

Binding affinities toward the nAChR and anticonvulsant potencies of α-substituted lactams and cyclic imides

#[a]	Compound	K_I for α3β4 nAChR (mM)[b]	ED_{50} in mice (mmol / kg of body weight, *i.p.*)[c]	
			MES	scPTZ
1	3,3-diethylpyrrolidin-2-one (DEP)	1.90 ± 0.04	1.23 (0.94-1.62)	0.33 (0.21-0.45)
2	3-isopropyl-3-methylpyrrolidin-2-one	1.75 ± 0.35	1.56 (1.24-2.12)	0.84 (0.49-1.36)
3	3-benzyl-3-ethylpyrrolidin-2-one	0.35 ± 0.02	0.36 (0.33-0.46)	0.21 (0.13-0.30)
4	3,3-diethylpiperidin-2-one	0.37 ± 0.07	0.35 (0.21-0.56)	0.24 (0.16-0.36)
5	hexahydro-3-ethyl-2H-azepin-2-one	3.24 ± 0.31	1.76 (1.32-2.36)	1.49 (1.22-1.82)
6	hexahydro-3,3-diethyl-2H-azepin-2-one	0.50 ± 0.01	1.05 (0.58-1.89)	1.33 (1.06-1.68)
7	3-ethyl-3-methylpyrrolidine-2,5-dione	7.43 ± 0.52	4.33 (3.86-4.80)	0.52 (0.43-0.62)
8	3-methyl-3-phenylpyrrolidine-2,5-dione	0.46 ± 0.02	0.22 (0.21-0.23)	0.30 (0.27-0.34)

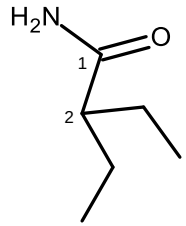
[a] The compounds are numbered according to Figure 3

[b] M ± SD; calculated using Equation (1) from the cell-flow data in the presence of 3-mM Carb (this study)

[c] 95% CI is given in the parentheses. ED_{50} values for 3-methyl-3-phenylpyrrolidine-2,5-dione were determined in this study. ED_{50} values for the lactams are from Reddy *et al.*[9,10], and ED_{50} values for 3-ethyl-3-methylpyrrolidine-2,5-dione (ethosuximide) are from Witiak *et al.*[33]

Table S2

Summary of molecular docking of 2,2-diethylacetamide (DEAA) to the AChBP (PDB ID: 1UV6)



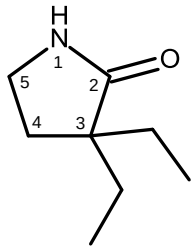
Score (kcal/mol)	RMSD[a]		Hydrogen bonds			Binding site location[b]
	l.b.	u.b.	Donor	Acceptor	Length, Å	
-4.1	0	0	Not appl.	Not appl.	Not appl.	Sub-agonist pocket
-4.1	22.1	22.7	NH_2 of DEAA	CO of Val83C	2.310	Vestibule pocket
-4.1	17.3	17.9	NH_2 of Gln54C	CO of DEAA	2.135	Vestibule pocket
			NH_2 of DEAA	CO of Leu86C	2.318	
-4.1	21.2	21.8	Not appl.	Not appl.	Not appl.	Vestibule pocket
-4.0	21.6	22.2	αNH of Val83C	CO of DEAA	2.335	Vestibule pocket
-4.0	1.84	3.07	Not appl.	Not appl.	Not appl.	Sub-agonist pocket
-4.0	4.09	5.25	ωNH_2 of Arg137C	CO of DEAA	2.471	Sub-agonist pocket
			ωNH_2 of Arg137C	CO of DEAA	1.930	
-3.9	3.77	4.73	NH_2 of DEAA	CO of Arg137C	2.219	Sub-agonist pocket
			NH_2 of Asn90C	CO of DEAA	2.488	
-3.9	28.9	29.5	NH_2 of DEAA	CO of Pro20C	1.840	Top pocket

[a] Root-mean-square deviation of ligand coordinates from the best docking mode; lower (l.b.) and upper (u.b.) bounds are given

[b] "sub-agonist pocket", "vestibule pocket", and "top pocket" refer to the nomenclature of the binding sites suggested by Ulens with collaborators[46] (see the Result and Discussion section of the main paper)

Table S3

Summary of molecular docking of 3,3-diethylpyrrolidin-2-one (DEP) to the AChBP (PDB ID: 1UV6)



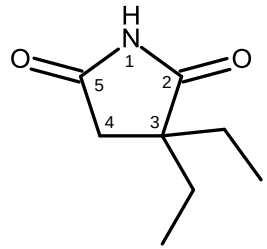
Score (kcal/mol)	RMSD[a]		Hydrogen bonds			Binding site location[b]
	l.b.	u.b.	Donor	Acceptor	Length, Å	
-4.9	0	0	Not appl.	Not appl.	Not appl.	Sub-agonist pocket
-4.8	1.53	3.00	Not appl.	Not appl.	Not appl.	Sub-agonist pocket
-4.6	28.6	29.8	Not appl.	Not appl.	Not appl.	Vestibule pocket
-4.6	21.7	23.0	αNH of Val83C	CO of DEP	2.444	Vestibule pocket
-4.6	21.9	23.2	αNH of Val83C	CO of DEP	2.161	Vestibule pocket
-4.6	28.2	29.4	Not appl.	Not appl.	Not appl.	Vestibule pocket
-4.5	28.1	29.2	αNH of Val83D	CO of DEP	2.473	Vestibule pocket
-4.5	21.5	22.8	Not appl.	Not appl.	Not appl.	Vestibule pocket
-4.5	21.9	23.2	NH of DEP	COOH of Asp17C	2.413	Vestibule pocket

[a] Root-mean-square deviation of ligand coordinates from the best docking mode; lower (l.b.) and upper (u.b.) bounds are given

[b] "sub-agonist pocket" and "vestibule pocket" refer to the nomenclature of the binding sites suggested by Ulens with collaborators[46] (see the Results and Discussion section of the main paper)

Table S4

Summary of molecular docking of 3,3-diethylpyrrolidine-2,5-dione (DEPD) to the AChBP (PDB ID: 1UV6)



Score (kcal/mol)	RMSD[a]		Hydrogen bonds			Binding site location[b]
	l.b.	u.b.	Donor	Acceptor	Length, Å	
-5.3	0	0	OH of Ser166D	CO of DEPD	2.501	Sub-agonist pocket
-5.3	1.01	3.19	Not appl.	Not appl.	Not appl.	Sub-agonist pocket
-5.3	22.2	23.1	Not appl.	Not appl.	Not appl.	Vestibule pocket
-5.2	21.1	23.0	αNH of Val83C	CO of DEPD	2.377	Vestibule pocket
-5.0	17.8	18.9	NH_2 of Gln54C	CO of DEPD	2.358	Vestibule pocket
-5.0	19.7	20.9	NH of DEPD	OH of Thr57D	2.370	Outer rim
-4.9	21.9	22.8	Not appl.	Not appl.	Not appl.	Vestibule pocket
-4.9	17.2	18.4	NH_2 of Gln54C	CO of DEPD	1.974	Vestibule pocket
			NH of DEPD	CO of Leu86C	2.068	
-4.9	21.3	22.2	αNH of Val83C	CO of DEPD	2.175	Vestibule pocket

[a] Root-mean-square deviation of ligand coordinates from the best docking mode; lower (l.b.) and upper (u.b.) bounds are given

[b] "sub-agonist pocket" and "vestibule pocket" refer to the nomenclature of the binding sites suggested by Ulens with collaborators[46] (see the Results and Discussion section of the main paper). "Outer rim" refers to a site in the upper part of the outer rim of the protein that does not resemble any of the previously proposed[4] sites

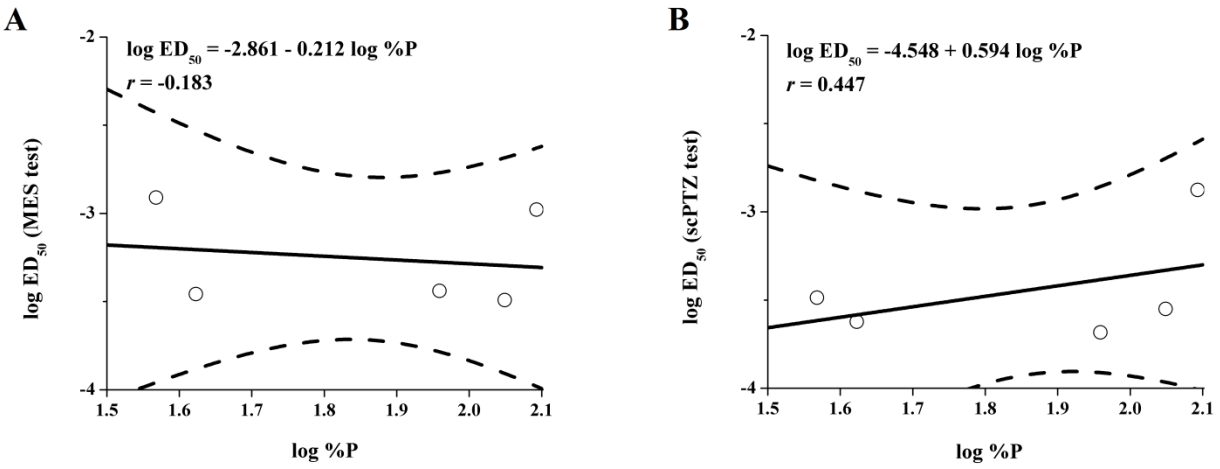


Figure S1: Anticonvulsant potency of α-substituted lactams does not correlate with their potentiation of the $GABA_A$ receptor.

Data pertaining to anticonvulsant potency in mice (mmol / kg of body weight) and potentiation of the $GABA_A$ receptor in cultured rat hippocampal neurons for 3,3-diethylpyrrolidin-2-one (DEP), 3-benzyl-3-ethylpyrrolidin-2-one, 3,3-diethylpiperidin-2-one, 3-benzyl-3-ethylpiperidin-2-one, and hexahydro-3,3-diethyl-2H-azepin-2-one were taken from the literature[9,10]. Potentiation (%P) was defined as $[(A_P/A_0)-1] \times 100\%$, where A_0 and A_P are the peak current amplitudes in the absence and presence of the potentiating compound. Error bars are omitted for clarity. The dashed lines are 95% confidence bands.

(**A**) No correlation between anticonvulsant potency in the MES test and potentiation of the $GABA_A$ receptor among the α-substituted lactams. The *P* value for the slope = 0 is 0.769.

(**B**) Poor correlation between anticonvulsant potency in the scPTZ test and potentiation of the $GABA_A$ receptor among the α-substituted lactams. The *P* value for the slope = 0 is 0.450.

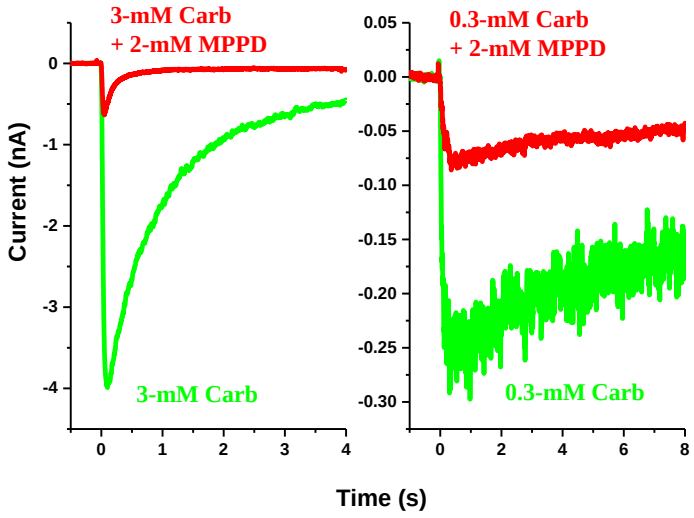


Figure S2: Inhibition of the α3β4 nAChR by 3-methyl-3-phenylpyrrolidine-2,5-dione (MPPD).

The experiments were conducted at room temperature (22-24 °C), pH 7.4, and - 60 mV. The cell-flow technique with a 20-ms time resolution[19] was used to record the current traces and to correct the current amplitudes for receptor desensitization. MPPD was co-applied with carbamoylcholine (Carb) in the extracellular buffer. The compositions of the buffers are given in the Methods section of the main paper.

Left panel: 4-s application of 3-mM Carb in the absence and in the presence of 2-mM MPPD.

Right panel: 8-s application of 0.3-mM Carb in the absence and in the presence of 2-mM MPPD.